PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

SCHLICH, George, William
Mathys & Squire
100 Gray's Inn Road
London WC1X 8AL
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 06.09.2004
Applicant's or agent's file reference GWS/CS/24346	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 03/03082	15.07.2003	19.07.2002

Applicant
HEALTH PROTECTION AGENCY et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Rauf, A Tel. +49 89 2399-7548

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference GWS/CS/24346	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/03082	International filing date *(day/month/year)* 15.07.2003	Priority date *(day/month/year)* 19.07.2002
International Patent Classification (IPC) or both national classification and IPC A61K47/48		
Applicant HEALTH PROTECTION AGENCY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28.01.2004	06.09.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Chavanne, F Telephone No. +49 89 2399-8399

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/03082

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-23 as originally filed

Claims, Numbers

1-22 received on 01.07.2004 with letter of 30.06.2004

Drawings, Sheets

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☒ the claims, Nos.: 23, 24

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-22
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-22
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations

   see separate sheet

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   D1: WO 00/28041
   D2: WO 01/58936

2. The subject-matter of claim 1 is not specifically disclosed in the prior art. Hence, said subject-matter is novel (Article 33(2) PCT).

3. The closest prior art to evaluate the inventiveness of the present application is either D1 or D2.
   The problem to be solved by the present application was to provide an improved composition for delivery of a therapeutic agent to neuronal cells.
   The solution provided by the present application is a composition comprising a therapeutic agent joined to a H_c domain of botulinum C_1 toxin.

   D1 describes a composition for delivery of a therapeutic agent to a neuronal cell comprising the therapeutic agent (superoxide dismutase) linked by a linker to a neuronal targeting component comprising a translocation domain and a receptor binding domain. The neuronal targeting component described in D1 comprises the neuronal cell binding domain (H_c domain) of botulinum A toxin. D1 mentions the use of the neuronal cell binding domain (H_c domain) of botulinum C_1 toxin in said composition. The translocation domains described in D1 are derived from clostridial sources. Methods for recombinantly making the composition comprising expressing a DNA that encodes the superoxide dismutase (SOD) and the neuronal cell targeting component is also disclosed in D1. D1 further mentions the use of said composition for promoting nerve regeneration (abstract; page 3, lines 26 and 27; page 4, line 3 to page 6, line 2; page 6, lines 15-21; page 7, lines 15-21; page 8, lines 5-13; page 9, lines 6-11; page 10, line 7 to page 11, line 20; page 12, line 32 to page 14; line 18; example 3; figures 3 and 4; claims 1-22).

   D2 describes compositions for the delivery of therapeutic agents to a neuronal cell. These compositions comprise a therapeutic agent linked to a neuronal targeting component comprising a H_c domain of botulinum F toxin, and a translocation domain which is derived from a non-clostridial source. This

translocation domain can be derived from e.g. the influenza virus, the diphteria toxin. D2 mentions the use of the neuronal cell binding domain (H_c domain) of botulinum C_1 toxin in said composition. Examples of therapeutic agents used in the composition of D2 include drugs, growth factors, enzymes, modified viruses, DNA. D2 also describes methods for recombinantly making said compositions, and mentions their use for the cure of nerve degeneration (abstract; page 1, lines 5-11; page 5, lines 1-24; page 6, lines 8-2; page 7, line 5 to page 8, line 16; page 10, line 9 to page 12 line 34; page 14, lines 10-27; page 15, lines 2-4; page 16, lines 9-36; figures 1 and 5; examples 1-6 and 9).

The subject-matter of claims 1-22 differs from the teachings of D1 or D2 in the type of therapeutic agent used. Moreover, compositions comprising a therapeutic agent linked to recombinantly made H_c domains of botulinum A, B or F toxins show a reduced binding affinity for neuronal cells compared to the native H_c domain (see e.g. page 3, lines 10-15; page 4, lines 9 and 10; appendix A and B). Compositions comprising a therapeutic agent linked to the recombinantly made H_c domain of botulinum C_1 toxin do not show such a reduced binding affinity for neuronal cells compared to the native H_c domain. This unexpected feature of the recombinantly made H_c domain of botulinum C_1 toxin, which allows the provision of improved compositions for delivery of a therapeutic agent to a neuronal cell could not be derived from the available prior art.
Thus, compositons for delivery of a therapeutic agent to a neuronal cell comprising a therapeutic agent **joined to** a neuronal cell targeting component **consisting** of a recombinantly made H_c domain of botulinum C_1 toxin, or a fragment thereof which retains **the binding affinity for neuronal cells** of the native H_c domain could be recognised inventive (Article 33(3) PCT).

4. The main problem of the present application is the clear definition of the claimed subject-matter (Article 6 PCT).

It appears from the present application that the therapeutic agent and the neuronal cell targeting component are joined to each other. According to the present wordings of claim 1, the claimed composition comprises these two components but they are not necessarily joined. Moreover, the neuronal cell targeting component consists of a H_c domain of botulinum C_1 toxin or a fragment thereof which retains the binding affinity for neuronal cells of native H_c domain. Due to the word "comprises", the scope of claim 1 encompasses a composition comprising a therapeutic agent and the whole botulinum C_1 toxin. The whole

botulinum C_1 toxin would not unexpectedly retain the binding affinity for neuronal cells of the native H_c domain, since it is the native H_c domain. Hence, the neuronal cell targeting component should be limited to the H_c domain of botulinum C_1 toxin, or a fragment thereof which retains the binding affinity for neuronal cells of native H_c domain. Moreover, the specific function of the H_c native domain (the binding affinity for neuronal cells) should be specified.

CLAIMS

1. A composition, for delivery of a therapeutic agent to a neuronal cell, comprising:

a therapeutic agent which inhibits at least one member of the Rho group of GTPases, and

a neuronal cell targeting component, which component comprises a H_c domain of botulinum C_1 toxin, or a fragment thereof which retains the function of the native H_c domain,

and wherein the H_c domain is made recombinantly.

2. A composition according to Claim 1 further comprising a domain for translocation of the therapeutic agent into a cell.

3. A composition according to Claim 2 wherein the translocation domain is derived from a clostridial source.

4. A composition according to Claim 2 wherein the translocation domain is derived from a non-clostridial source.

5. A composition according to Claim 3 wherein the translocation domain is derived from *C. botulinum, C. butylicum, C. argentinense* or C. *tetani.*

6. A composition according to Claim 4 wherein the translocation domain is derived from diphtheria toxin, *Pseudomonas* exotoxin A, influenza virus haemagglutinin fusogenic peptides or amphiphilic peptides.

7. A composition according to Claim 2, wherein the translocation domain is derived from botulinum C_1 toxin and fragments, variants and derivatives thereof, or diphtheria toxin and fragments, variants and derivatives thereof.

8. A composition according to Claim 2 wherein the translocation domain is a membrane disrupting peptide.

9. A composition according Claim 1, wherein the therapeutic agent is selected from the group consisting of drugs, growth factors, enzymes, DNA, modified viruses, drug release systems, or a combination thereof.

10. A composition according to any preceding claim wherein the therapeutic agent is a C3 enzyme.

11. A composition according to Claim 10, wherein the C3 enzyme is derived from *C. botulinum, C. limosum, B. cereus, S. aureus, C. acetobutylicum, S. pyogenes, L. monocytogenes.*

12. A composition according to Claim 10 wherein the C3 enzyme is selected from the group consisting of C3Stau2, C3Stau1, and C3bot.

13. A composition according to Claim 10 wherein the C3 enzyme is selected from SEQ ID Nos: 1-10.

14. A composition according any preceding claim, wherein the therapeutic agent and the H_c domain are joined to each other directly or via a linker molecule.

15. A composition according to any of Claims 2-13 wherein the therapeutic agent, the Hc domain and the translocation domain are joined to each other directly or via a linker molecule.

16. A composition according to Claim 14 or 15, wherein the linker molecule is selected from the group consisting of $(GGGGS)_2$, $(GGGGS)_3$, the interdomain linker of cellulase, PPPIEGR, collagen-like spacer, trypsin-sensitive diphtheria toxin peptide, or SEQ ID Nos: 16-24.

17. A composition according to any preceding claim wherein the composition is a single polypeptide.

18. A composition according to any of Claims 1-16 , wherein the composition is a dichain polypeptide.

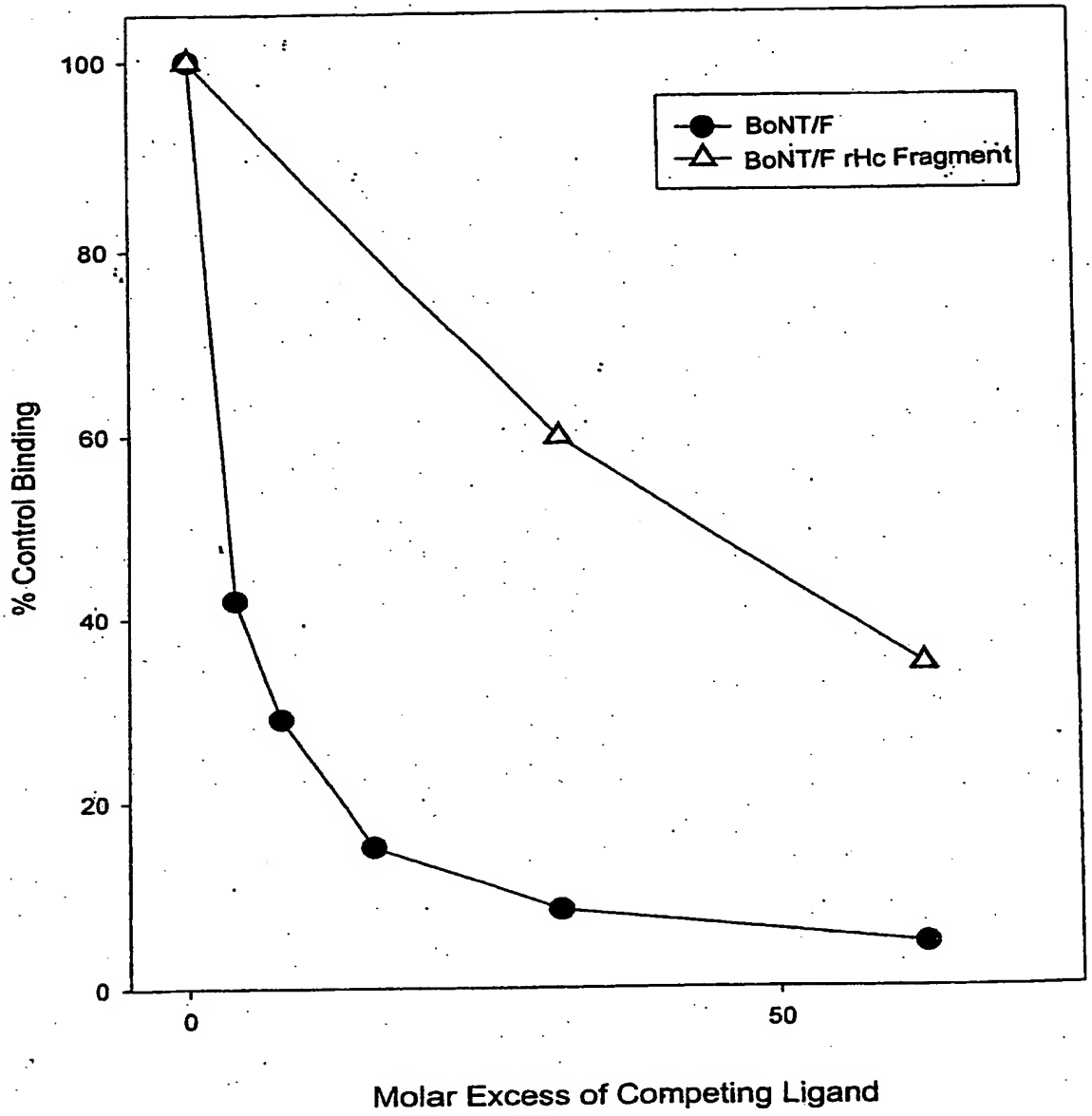
19. A composition according to any preceding claim, wherein the composition is a suspension, emulsion, solution or a freeze-dried powder.

20. A composition according to any preceding claim, wherein the construct of the invention is re-suspended or diluted in a pharmaceutically acceptable liquid.

21. A method of making a composition of the invention according to any of Claims 1-20 comprising expressing a DNA encoding the therapeutic agent and the neuronal cell targeting domain.

22. Use of the composition of any of Claims 1-20 for the manufacture of a medicament for promoting nerve regeneration.

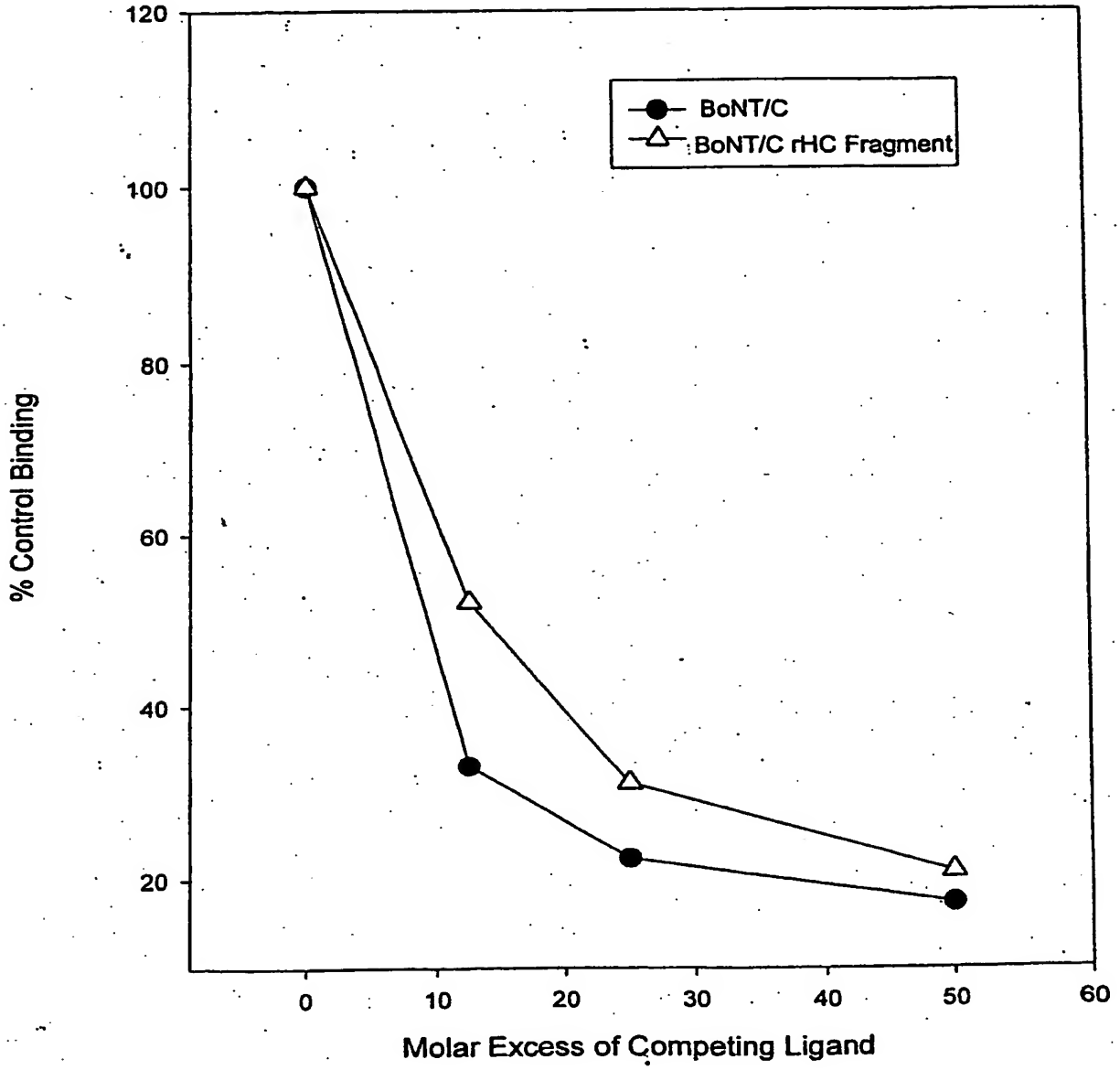
Appendix A



Legend

This Figure shows the ability of cold ligand (either neurotoxin or its rHc fragment) to compete with ^{125}I-labelled neurotxin for receptors on rat brain synaptosomes.

Appendix B



Legend

This Figure shows the ability of cold ligand (either neurotoxin or its rHc fragment) to compete with ^{125}I-labelled neurotxin for receptors on rat brain synaptosomes.